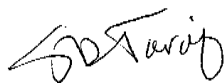


January 19, 2006

To: Marian Stanley, ACC

From: Suzette Tardif, Ph.D.



Subject: Findings regarding female reproductive physiology from the Mitsubishi Study #B000496, "Sixty-five week repeated oral dose toxicity study of DEHP in juvenile common marmosets"

I have reviewed the findings from the above-referenced study relative to female reproductive physiology – specifically the findings relative to ovarian weight at the end of the study and to circulating estradiol concentrations throughout the study. I do not believe that these findings can be used to support or refute the conclusion that DEHP treatment resulted in precocious puberty in female marmosets, primarily due to the extremely low body weights of the majority of the subjects. My reasoning is as follows:

1. The weights of female subjects at the end of the study (week 66 of study, when the animals would be approximately 78 weeks, or 17 months, old) is extremely low, particularly for the control group and for females receiving the lowest DEHP dose (100) – see Figure 1. The average weights for females in the four groups are all well below the average for a healthy marmoset at 17 months of age (350-370 grams). I believe this reflects the fact that the study procedures, involving daily gavage for many weeks in a row, resulted in many basically unhealthy animals with impaired growth.

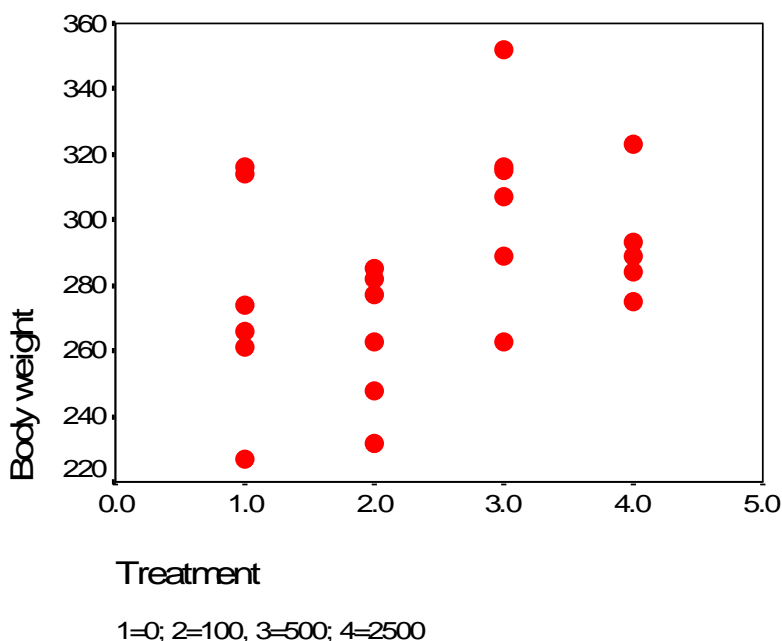


FIGURE 1.

2. While the difference in average weights across the treatment groups is not significant at $p < 0.05$ ($F = 2.849$, $p = 0.065$), there is a trend for females in the two highest DEHP doses to have higher weights (see Figure 1). In our colony, we would consider any 17 month old animal below 275 grams as unsuitable for research use. If we use this criterion on the Mitsubishi study population, then only 3 out of 6 subjects in the control and low dose groups would be suitable, while 5/6 and 5/5 of the animals in the higher dose groups would be suitable.

3. Estradiol concentrations appear to be bimodally distributed, with most under 50pg/ml and a few from 130-1300 pg/ml. I believe this bimodal distribution reflects the difference between pre-pubertal and post-pubertal state. The range for earliest age at which subjects displayed estradiol concentrations reflective of likely post-pubertal state is from around 51 weeks (or 12 months) of age to 65 weeks (or 17 months) of age. The lower limit on this range (12 months of age) is well within the norm for age of puberty for female marmosets. Animals who appear to remain prepubescent up to 17 months of age ($n=5$) and who appear to be pre-pubescent throughout the length of the study ($n=11$) are abnormal, displaying what I would call delayed puberty. This condition appears to be related to body weight – see Figure 2

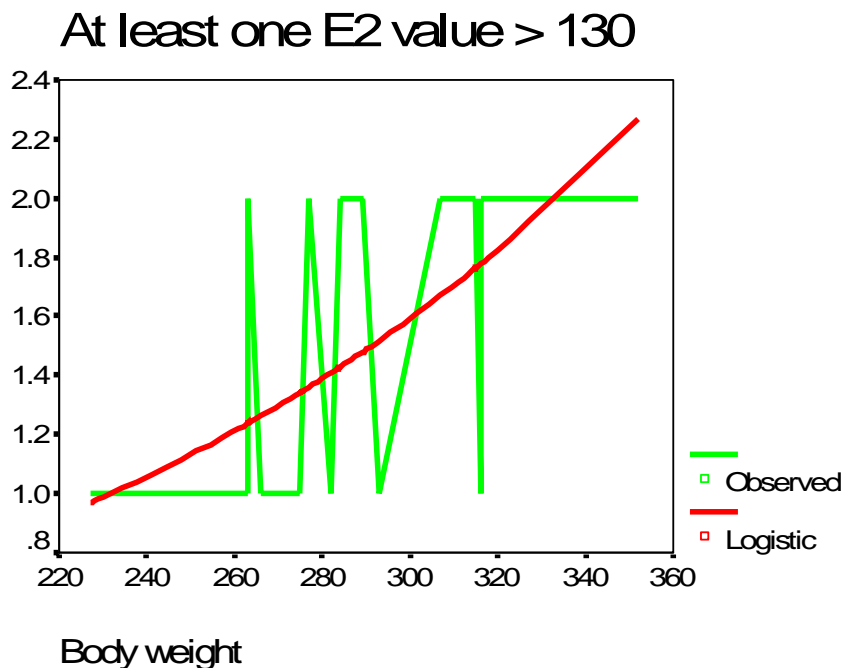


Figure 2. Logistics regression of puberty occurrence (as assessed by at least one estradiol concentration over 130 pg/ml; 1=no; 2=yes) versus body weight. Body weight was a significant determinant of puberty occurrence ($F = 10.64$, $df = 21$, $p = 0.004$).

4. Ovarian weight is largely reflective of body weight. Relative ovarian weight (ovarian weight/body weight) is also still largely reflective of body weight – see Figure 3.

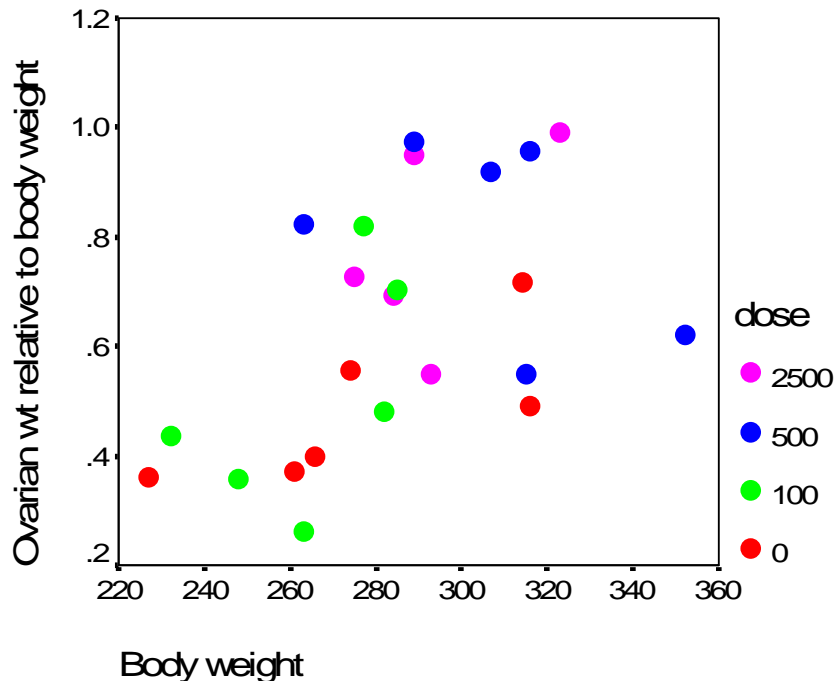


Figure 3.

In a general linear model analysis of relative ovarian weight x treatment, with body weight as a covariate, there is a marginal relationship between treatment and relative ovarian weight ($F=2.787$, $df=3$, $p=0.070$). If those subjects below 275 grams are removed (see #2), then there is no indication of a relation between treatment and relative ovarian weight ($F=1.097$, $df=3$, $p=0.391$), however the sample size is so small for the control and low dose group that the validity of the comparison becomes questionable.

The higher ovarian weights were generally associated with higher body weights and occurrence of ovulation/corpus luteum formation – i.e., they are normal in a sexually mature female. The fact that these higher ovarian weights were seen in the group with the highest dose exposure is, I believe, simply due to the fact that this group contained more normal weight animals who, therefore, were more likely to have ovulated. Normal ovarian function in marmosets includes the development and maintenance of a large, steroidogenic interstitial gland. The persistent presence of this gland, along with the cyclical presence of corpus lutea, means that sexually mature females will have higher ovarian weights. I disagree with the study conclusion that such features are “usually observed in more mature females.” At the age at which these females were sacrificed, I would have expected most, if not all, of them to display evidence of sexual maturity.